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INHIBITION OF BACTERIAL VIRUSES (BACTERIOPHAGE) BY CERTAIN SUBSTANCES

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The problem of autogenesis of a virus has acquired special significance since it was discovered that many filterable viruses are unique albuminous substances. While the question of autogenesis of virus molecules has only recently become an object of theoretical discussion abroad, we have tried since 1938 to arrive at an experimental solution of the problem and have systematically studied the physiological conditions in the reproduction of virus albumin which causes tobacco mosaic. One of the methods of studying this type is to study the accumulation of a virus with one of the fermentative systems inhibited. Work in this direction led us to the discovery of inhibitors of reproduction of virus albumin, as well as to the establishment of the independence of this reproduction from the glycolytic system of the cell.

Similar questions were undertaken with regard to bacterial viruses in 1943 independently of our studies. It seemed that some substances, such as dinitrophenol, which according to our data inhibited the virus of tobacco mosaic, also inhibited the reproduction of the virus which affects Bac coli. The works of Fitzgerald and Babbitt are of particular interest from the point of view of this parallelism. These authors discovered that an increase of B. coli phage is inhibited by acridine preparations and especially by acriflavine. Rivanol, which is similar to acriflavine, was especially effective in our experiments with the virus of tobacco mosaic. Therefore, these studies showed that the virus of tobacco mosaic and bacterial viruses

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that differ greatly from it depend upon similar if not identical conditions in their reproduction. These discoveries present a hope of penetrating the mechanism of reproduction of a virus molecule and of finding preparations which would be valuable in the chemoprophylaxis and chemotherapy of virus diseases.

Considering the importance of these prospects we undertook a study which was to verify and expand the work cited above on bacterial viruses. Using Fitzgerold and Babbitt's method, we observed the inhibition of bac coli phage by rivanol and trypaflavine in a concentration of 0.5 mg% and by acridine in a dilution of 4 mg%. Green malachite inhibited phage in the identical concentrations as rivanol. All of these substances had only a slight effect on the reproduction of bacteria. Monoiodacetic acid inhibited the reproduction of phage only in concentrations which also inhibited the reproduction of bacteria. We also checked the action of rivanol on phages which were not studied by Fitzegerold. The bacterial viruses affecting Bact typhi abdominalis and Vibrio cholerae asiaticae are inhibited by rivanol if they are introduced into a culture in a dilution of 1:100—1:1,000, while phage Bact. dysenteriae Flexner is inhibited by rivanol only if the phage is introduced into a culture in high dilution(1:1 million). This indicates a difference in the sensitivity of various bacterial viruses to rivanol.

The Spizizen method was used for quantitative study of the influence of some substances on the reproduction of Bact. coli phage. The results are presented in Table 1.

Table 1. Influence of Some Substances on the Accumulation of Phage
(Number of particles of phage in 1 cc)

Substance		Test	Control	Duration of Experiment in hours
Para-amidophenol	0.1%	6,000	2,660,000	1½
Thiamin	1%	2,000	5,000,000	1½
Dinitrophenol	4mg%	6,000	120,000	1½
Sodium fluoride	0.168%	2,740,000	5,400,000	1½
Rivanol	0.5mg%	28,600	80,000,000	3

It is seen from Table 2 that the substances whose action we studied also acted on the growth of bacteria to some degree. Calculation of the quantity of bacteria was carried out by filtering the bacterial suspension on a solid culture medium and calculating the colonies.

Table 2. Influence of Inhibitors of a Phage on Reproduction
(Number of bacteria in 1 cc in millions)

Substance		Test	Control	Duration of Experiment in hours
Para-amidophenol	0.1%	200,000	4,700,000	1½
Thiamin	1%	80,000	2,600,000	1½
Dinitrophenol	4mg%	1,400,000	12,200,000	1½
Sodium fluoride	0.0168%	6,560,000	15,200,000	1½
Rivanol	0.5mg%	35,000,000	71,000,000	3

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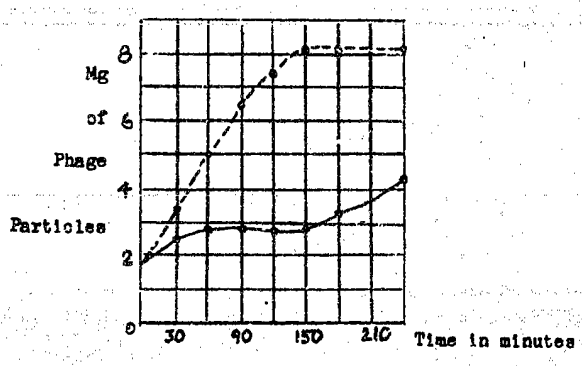
It is seen from Table 2 that dinitrophenol, thiamin, and para-amidophenol also effectively inhibit the reproduction of bacteria in concentrations which act on the reproduction of phage and affects the growth of bacteria only very slightly. This is seen especially in a comparison of the action of sodium fluoride and rivanol. Both of these substances inhibit the growth of bacteria in approximately the same degree, but sodium fluoride has almost no influence on the reproduction of phage while rivanol inhibits it. This permits us to speak of the more specific action of an acridine preparation on the reproduction of bacterial viruses. A logarithmic curve of the accumulation of phage in the control and in the test with rivanol is presented in the figure.



——— B. Coli + phage + rivanol

– – – B. Coli - phage

Our experiments further showed that if the rivanol solution is not introduced into the culture simultaneously with the phage as Fitzgerold and Babbitt did, but introduced after one-half hour to one hour, the inhibitive action on the lysis is still retained. When the rivanol is introduced after 1½ hours it shows no or almost no action.

Fitzgerold and Babbitt showed that yeast nucleinic acid lowers the action of rivanol on phage in a manner similar to the way it lowers the action of acridine preparations on bacteria. We can confirm this effect of yeast nucleinic acid. Nucleinic acid shows its antagonistic influence if it is introduced simultaneously with rivanol. When it is introduced a half hour after the rivanol the effect of the acid is already weakened, and when it is introduced an hour later the effect is completely unnoticeable. It can be supposed that the action of the nucleinic acid depends upon the fact that it forms a compound with the rivanol which does not penetrate or poorly penetrates the bacterial cell.

There is no doubt that all the substances about whose capacity to inhibit autogenesis of bacterial viruses we have spoken here have no direct toxic effect on these viruses in the concentrations used.

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